FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)
208888US0PCT	09/831907

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR99/02941	**26 November 1999**	**26 November 1998**

TITLE OF INVENTION

MAMMALIAN UROTENSINS II AND APPLICATIONS THEREOF

APPLICANT(S) FOR DO/EO/US

BEAUVILLAIN Jean-Claude et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☒ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Notice for Consideration of Documents Cited in International Search Report/Notice of Priority/PCT/IB/304
Amended Sheets (Pages 16, 17, 18, 19 and 20)/PCT/IB/308/Sequence Listing (10 Sheets)/Drawings (8 Sheets)

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/831907	PCT/FR99/02941	208888US0PCT

24. The following fees are submitted:.		CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :			
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1000.00**		
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	**$860.00**		
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$710.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$690.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =		**$860.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		**$130.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	- 20 =	0	x $18.00	**$0.00**	
Independent claims	- 3 =	0	x $80.00	**$0.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$990.00**	
☐ Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$0.00**	
SUBTOTAL =				**$990.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$990.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**	
TOTAL FEES ENCLOSED =				**$990.00**	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of **$990.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **15-0030** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

22850

Surinder Sachar
Registration No. 34,423

Surinder Sachar
SIGNATURE

Norman F. Oblon
NAME

24,618
REGISTRATION NUMBER

May 25 2001
DATE

208888US-0PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :

JEAN-CLAUDE BEAUVILLAIN : ATTN: APPLICATION DIVISION

SERIAL NO: 09/831,907 :

FILED: MAY 25, 2001 :

FOR: MAMMALIAN UROTENSINS II AND APPLICATIONS THEREOF :

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please amend the above-identified application as follows:

IN THE SPECIFICATION

Please delete the original Sequence Listing.

Page 20 (Abstract), after the last line, beginning on a new page, please insert the attached substitute Sequence Listing.

IN THE CLAIMS

Please amend the claims as shown on the marked-up copy following this amendment to read as follows.

1. (Amended) A polypeptide isolated from mammals, characterized in that it comprises, at its C-terminal end, a heptapeptide having the following sequence: Cys-Phe,Trp-Lys-Tyr-Cys-Xaa, in which Xaa represents Val or Ile, in that it belongs to the urotensin II family and in that it exhibits at least 45% similarity with the polypeptide sequence SEQ ID NO:1, corresponding to human prepro-urotensin II.

3. (Amended) A purified nucleic acid fragment, characterized in that it is selected from the group consisting of:

a) the fragments comprising at least one sequence encoding a polypeptide as claimed in claim 1,

b) the fragments consisting of a sequence encoding a polypeptide as claimed in claim 1,

c) the oligonucleotides derived from the sequences as defined in b), constituting probes or primers, and

d) the sequences complementary to the above sequences, which may be sense or antisense sequences, with the exception of the EST having the Gen Bank accession number AA535545.

5. (Amended) A recombinant vector, characterized in that it contains a nucleic acid fragment as claimed in claim 3.

6. (Amended) A cell transformed with at least one nucleic acid fragment as claimed in claim 3.

7. (Amended) A reagent for detecting a nucleic acid fragment as claimed in claim 3, characterized in that it comprises between 20 and 50 nucleotides of the sequence SEQ ID NO:4, of the sequence SEQ ID NO:18 or of the sequence SEQ ID NO:27.

9. (Amended) A pharmaceutical composition, characterized in that it comprises at least one polypeptide isolated from mammals, characterized in that it comprises, at its C-terminal end, a heptapeptide having the following sequence: Cys-Phe,Trp-Lys-Tyr-Cys-Xaa, in which Xaa represents Val or Ile, in that it belongs to the urotensin II family and in that it exhibits at least 45%, and preferably at least 70%, similarity with the polypeptide sequence SEQ ID NO:1, corresponding to human prepro-urotensin II, or one nucleic acid sequence as claimed in claim 3 encoding all or part of said polypeptides, combined with at least one pharmaceutically acceptable vehicle.

11. (Amended) A process for detecting the presence or absence of an mRNA encoding a mammalian urotensin II, in particular in individuals with a neurodegenerative pathology or a trauma to the spinal cord, by bringing a biological sample into contact with at least one reagent as claimed in claim 7.

12. (Amended) A process for detecting a mutation in the sequence of the gene or of the mRNA encoding urotensin, characterized in that it comprises extracting said DNA or said mRNA from a biological sample and comparing it with the nucleic acid sequences as claimed in claim 3.

13. (Amended) A diagnostic kit intended for detecting an mRNA encoding a mammalian urotensin II, in a biological sample, said mRNA possibly being mutated, characterized in that it comprises at least one sequence as claimed in claim 3.

14. (Amended) A method for selecting anti-hypertensives comprising determining the activity of an anti-hypertensive against urotensin II as an antagonist.

Please add the following new claims.

15. (New) The polypeptide claimed in claim 1 wherein said polypeptide exhibits at least 70% similarity with the polypeptide sequence SEQ ID NO:1, corresponding to human prepro-urotensin II.

REMARKS

Claims 1-15 are active in the present application. Claims 3, 5-7 and 9-13 have been amended to remove multiple dependencies. Claims 1 and 14 have been rewritten to conform to U.S. Patent Practice. Claim 15 is a new claim. Support for amended claim 14 is found in the specification on page 10, lines 21-25. No new matter is added.

Applicants have now submitted a substitute Sequence Listing and a corresponding computer-readable Sequence Listing. The sequence information recorded in the corresponding computer-readable Sequence Listing is identical to the paper copy of the substitute Sequence Listing. Support for all of the sequences listed in the substitute Sequence Listing is found in the present application as originally filed. No new matter is believed to have been introduced by the submission of the substitute Sequence Listing and the corresponding computer-readable Sequence Listing.

Applicants submit that the present application is ready for examination on the merits.

Early notice to this effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Norman F. Oblon
Attorney of Record
Registration No. 24,618

Daniel J. Pereira, Ph.D.
Registration No. 45,518

22850

Tel.: (703) 413-3000
Fax: (703) 412-2220
NFO:DJP:SUKOS\la
D:\208888US0PCT-PrA-seq.wpd

Marked-Up Copy
Serial No:09/831,907
Amendment Filed on:

IN THE CLAIMS

Please amend the claims as shown on the marked-up copy following this amendment to read as follows.

-- 1. (Amended) A polypeptide isolated from mammals, characterized in that it comprises, at its C-terminal end, a heptapeptide having the following sequence: Cys-Phe,Trp-Lys-Tyr-Cys-Xaa, in which Xaa represents Val or Ile, in that it belongs to the urotensin II family and in that it exhibits at least 45%[, and preferably at least 70%,] similarity with the polypeptide sequence SEQ ID NO:1, corresponding to human prepro-urotensin II.

3. (Amended) A purified nucleic acid fragment, characterized in that it is selected from the group consisting of:

a) the fragments comprising at least one sequence encoding a polypeptide as claimed in claim 1 [or claim 2],

b) the fragments consisting of a sequence encoding a polypeptide as claimed in claim 1 [or claim 2],

c) the oligonucleotides derived from the sequences as defined in b), constituting probes or primers, and

d) the sequences complementary to the above sequences, which may be sense or antisense sequences, with the exception of the EST having the Gen Bank accession number AA535545.

5. (Amended) A recombinant vector, characterized in that it contains a nucleic acid fragment as claimed in claim 3 [or claim 4].

6. (Amended) A cell transformed with at least one nucleic acid fragment as claimed in claim 3 [or claim 4].

7. (Amended) A reagent for detecting a nucleic acid fragment as claimed in claim 3 [or claim 4], characterized in that it comprises between 20 and 50 nucleotides of the sequence SEQ ID NO:4, of the sequence SEQ ID NO:18 or of the sequence SEQ ID NO:27.

9. (Amended) A pharmaceutical composition, characterized in that it comprises at least one polypeptide isolated from mammals, characterized in that it comprises, at its C-terminal end, a heptapeptide having the following sequence: Cys-Phe,Trp-Lys-Tyr-Cys-Xaa, in which Xaa represents Val or Ile, in that it belongs to the urotensin II family and in that it exhibits at least 45%, and preferably at least 70%, similarity with the polypeptide sequence SEQ ID NO:1, corresponding to human prepro-urotensin II [as claimed in either of claims 1 and 2], or one nucleic acid sequence as claimed in [either of claims 3 and 4] claim 3 encoding all or part of said polypeptides, combined with at least one pharmaceutically acceptable vehicle.

11. (Amended) A process for detecting the presence or absence of an mRNA encoding a mammalian urotensin II, in particular in individuals with a neurodegenerative pathology or a trauma to the spinal cord, by bringing a biological sample into contact with at least one reagent as claimed in claim 7 [or claim 8].

12. (Amended) A process for detecting a mutation in the sequence of the gene or of the mRNA encoding urotensin, characterized in that it comprises extracting said DNA or said mRNA from a biological sample and comparing it with the nucleic acid sequences as claimed in claim 3 [or claim 4].

13. (Amended) A diagnostic kit intended for detecting an mRNA encoding a mammalian urotensin II, in a biological sample, said mRNA possibly being mutated, characterized in that it comprises at least one sequence as claimed in [either of claims 3 and 4] claim 3.

14. (Amended) [The use of the polypeptides as claimed in claim 1 [or claim 2], for selecting anti-hypertensives.] A method for selecting anti-hypertensives comprising determining the activity of an anti-hypertensive against urotensin II as an antagonist. --

Claim 15 (New).

MAMMALIAN UROTENSINS II AND APPLICATIONS THEREOF

The present invention relates to mammalian polypeptides, in particular of human or murine origin, having a structure of the urotensin II (UII) type (prepro-urotensin II, pro-urotensin II and urotensin II), and also to applications thereof as a medicinal product, in particular in the form of a composition intended for the treatment of neurodegenerative diseases of traumas to the spinal cord (hemiplegia, paraplegia), and as a tool for screening antihypertensive medicinal products.

The present invention also relates to nucleic acid sequences encoding said polypeptides, to oligonucleotides included in said sequences, and to the use of said sequences as primers and as probes, or for expressing mammalian urotensins II, and in particular human or murine urotensin II.

Urotensin II is a neuropeptide which was first characterized in the urophysis of teleost fish. In these fish, urotensin II is a cyclic peptide comprising 12 amino acids. The characterization of urotensin II in several species of teleost fish has shown that the structure of the C-terminal cyclic heptapeptide is conserved, whereas substitutions are observed in the N-terminal portion of the molecule. This heptapeptide has the following sequence: Cys-Phe-Trp-Lys-Tyr-Cys-Val (SEQ ID NO:9) (1-3).

For many years, it was thought that this peptide was produced exclusively in the urophysis of teleost fish (3), a small neurohemal organ exhibiting similarities with the neurohypophysis, located at the caudal end of the spinal cord; however, it has become apparent that this neuropeptide is not restricted to the caudal neurosecretory system of the fish. It has also been isolated from extracts of trout, skate (4) or lamprey (5) brain. In addition, a peptide similar to fish urotensin II has been detected in the central

nervous system (CNS) of the frog (*Rana ridibunda*) (6) and in a gastropod (*Aplysia californica*) in the cerebral ganglion (7).

This peptide, which, in the frog, comprises 13 amino acids, exhibits structural similarities with fish urotensins II, and in particular the cyclic region containing the abovementioned heptapeptide.

This neuropeptide also exhibits similarities with somatostatin (2,3); however, fish urotensin II has essentially cardiovascular effects, which can also be observed when this urotensin is administered to mammals, such as rats or rabbits (8,9): contractile effect on arteries (action observed in rats (8) and rabbits (10)), contraction of smooth muscles (spasmogenic effect on certain smooth muscles (bladder and ileum) in amphibians (11)) and effects on cardiac rhythm (observed in amphibians (12)).

It has also been shown that fish urotensin II is expressed in the form of precursors, the primary structures of which have been determined using the caudal neurosecretory system of the carp (Cyprinus carpio) (13).

The inventors have found, unexpectedly, that a urotensin II is expressed in mammals, in particular in humans and in mice, and that, in humans, it can have an activity on motoneuron survival and/or regeneration and on arterial blood pressure (hypertension).

A subject of the present invention is polypeptides, isolated from mammals, characterized in that they comprise, at their C-terminal end, a heptapeptide having the following sequence: Cys-Phe,Trp-Lys-Tyr-Cys-Xaa, in which Xaa represents Val or Ile, in that they belong to the urotensin II family and in that they exhibit at least 45%, and preferably at least 70%, similarity with the polypeptide sequence SEQ ID NO:1, corresponding to human prepro-urotensin II.

The similarity is quantified using the Clustal® program, which is in particular available over the Internet (site http://www2.ebi.ac.uk/clustalw/).

The present invention encompasses in particular:

- human prepro-urotensin II (SEQ ID NO:1), human pro-urotensin II (SEQ ID NO:2) and human urotensin II (SEQ ID NO:3),

- rat prepro-urotensin II (SEQ ID NO:30), rat pro-urotensin II (SEQ ID NO:31) and rat urotensin II (SEQ ID NO:32),

- mouse prepro-urotensin II (SEQ ID NO:33), mouse pro-urotensin II (SEQ ID NO:34) and mouse urotensin II (SEQ ID NO:35).

These mammalian polypeptide sequences exhibit, overall, a slight similarity with the fish or frog sequences (Figure 1 and Figure 4):

- 16% similarity between carp prepro-UII-α or prepro-UII-γ and human prepro-II;

- 25% similarity between frog prepro-UII and human prepro-UII.

At the N-terminal of human UII, these sequences exhibit no similarity with the nonmammalian UIIs previously described.

The invention also encompasses polypeptides or peptides derived from mammalian urotensins II and from precursors thereof, according to the invention, by the addition, deletion or substitution of one or more amino acids; they may, for example, be polypeptides into which modifications have been introduced, in particular by substituting dextrorotatory amino acids with levorotatory amino acids (pseudopeptides), or polypeptides which are obtained by molecular modeling and which have urotensin II activity at the neuromuscular junction or other biological targets for urotensin II.

A subject of the present invention is also a purified nucleic acid fragment, characterized in that it comprises all or part of a sequence encoding a

mammalian urotensin II as defined above, or of the sequence complementary thereto, which may be a sense or antisense sequence, with the exception of the EST having the Gen Bank accession number AA535545.

In this context, the present invention in particular encompasses the cDNAs, mRNAs and genomic DNAs of the urotensins II and of precursors thereof.

It encompasses the following sequences:

* human sequences:

- the sequence encoding human prepro-urotensin II, of sequence SEQ ID NO:4, which comprises 551 bp and in which:

. segment 1-32 is a noncoding sequence,

. segment 33-407 encodes human prepro-urotensin II, segment 33-92 corresponding to the sequence encoding the signal peptide, and

. segment 408-551 is noncoding (see Figure 2);

- a fragment of the sequence encoding human prepro-urotensin II (sequence SEQ ID NO:5), characterized in that it encodes human pro-urotensin II, the precursor of human urotensin II, and corresponds to segment 93-407 of SEQ ID NO:4;

- a fragment of the sequence encoding human prepro-urotensin II (sequence SEQ ID NO:6), characterized in that it encodes human urotensin II and corresponds to segment 372-407 of the sequence SEQ ID NO:4;

- a fragment of the sequence encoding human prepro-urotensin II, which encodes a dipeptide (Pro-Tyr), and which is upstream of the tribasic cleavage site, itself located just upstream of the sequence encoding human urotensin II and specific for said human sequence (see Figure 2);

- fragments which can be used as primers consisting of 20 to 50 nucleotides of SEQ ID NO:4, and in particular the sequences SEQ ID NO:7-8 and 10-17, and more particularly the following primer pairs:

. the sequences SEQ ID NO:7 and NO:8, corresponding to segments 267-292 and 535-511, respectively, of the sequence SEQ ID NO:4;

. the sequences SEQ ID NO:10 and 11, corresponding to positions 198-216 and 381-404, respectively, of the sequence SEQ ID NO:4;

. the sequences SEQ ID NO:12 and 13, corresponding to positions 46-65 and 214-195, respectively of the sequence SEQ ID NO:4;

. the sequences SEQ ID NO:14 (positions 9-28 of the sequence SEQ ID NO:4) and SEQ ID NO:13;

. the sequences SEQ ID NO:15 (positions 14-33 of the sequence SEQ ID NO:4) and SEQ ID NO:13;

. the sequences SEQ ID NO:12 and SEQ ID NO:16 (positions 150-131 of the sequence SEQ ID NO:4);

. the sequences SEQ ID NO:17 (positions 8-27 of the sequence SEQ ID NO:4) and SEQ ID NO:13;

- fragments which can be used as probes: sequence SEQ ID NO:4 and the fragments consisting of 20 to 50 nucleotides of the sequence SEQ ID NO:4. Said probes are preferably used under the following hybridization conditions:

. hybridization: 5X SSPE (0.9 M NaCl/0.05 M sodium phosphate buffer, pH 7.7/0.005 M EDTA), 0.1% SDS, 10X Denhardt's (0.2% Ficoll/0.2% polyvinylpyrrolidone/0.2% BSA), 50 µg/ml tRNA, 50 µg/ml denatured salmon sperm DNA. 37°C, overnight.

. washes: 5X SSPE/0.1% SDS, 4 times 5 minutes, room temperature, 3X SSPE/0.1% SDS, 2 times 10 minutes, 30°C.

* rat sequences:

- the sequence encoding rat prepro-urotensin II, of sequence SEQ ID NO:18, which comprises 529 bp and in which:

. segment 1-36 is a noncoding sequence,

. segment 37-405 encodes rat prepro-urotensin II, segment 37-96 corresponding to the sequence encoding the signal peptide, and

. segment 406-529 is noncoding (see Figure 3);

- a fragment of the sequence encoding rat prepro-urotensin II (sequence SEQ ID NO:19), characterized in that it encodes rat pro-urotensin II, the precursor of rat urotensin II, and corresponds to segment 96-405 of the sequence SEQ ID NO:18;

- a fragment of the sequence encoding rat prepro-urotensin II (sequence SEQ ID NO:20), characterized in that it encodes rat urotensin II and corresponds to segment 364-405 of the sequence SEQ ID NO:18;

- fragments which can be used as primers consisting of 20 to 50 nucleotides of SEQ ID NO:18, and in particular the sequences SEQ ID NO:36-42, and more particularly the following pairs of primers:

. the sequences SEQ ID NO:36 and SEQ ID NO:37, corresponding to positions 295-314 and 504-485, respectively, of the sequence SEQ ID NO:18;

. the sequences SEQ ID NO:38 (positions 280-299 of the sequence SEQ ID NO:18) and SEQ ID NO:37;

. the sequences SEQ ID NO:39 (positions 131-150 of the sequence SEQ ID NO:18) and SEQ ID NO:40 (positions 314-295 of SEQ ID NO:18);

. the sequences SEQ ID NO:41 (positions 322-341 of the sequence SEQ ID NO:18) and SEQ ID NO:37;

. the sequences SEQ ID NO:42 (positions 50-69 of SEQ ID NO:18) and SEQ ID NO:40;

- fragments which can be used as probes: sequence SEQ ID NO:18 and the fragments consisting of 20 to 50 nucleotides of the sequence SEQ ID NO:18, in particular SEQ ID NO:43 (positions 192-221 of the sequence SEQ ID NO:18).

* mouse sequences

- the sequence encoding mouse prepro-urotensin II, of sequence SEQ ID NO:27, which comprises 539 bp and in which:

. segment 1-36 is a noncoding sequence,

. segment 37-405 encodes mouse prepro-urotensin II, segment 37-96 corresponding to the sequence encoding the signal peptide, and

. segment 406-539 is noncoding (see Figure 4);

- a fragment of the sequence encoding mouse prepro-urotensin II (SEQ ID NO:28), characterized in that it encodes mouse pro-urotensin II, the precursor of mouse urotensin, and corresponds to segment 97-405 of SEQ ID NO:27;

- a fragment of the sequence encoding mouse prepro-urotensin II (sequence SEQ ID NO:29), characterized in that it encodes mouse urotensin II and corresponds to segment 355-405 of the sequence SEQ ID NO:27;

- fragments which can be used as primers consisting of 20 to 50 nucleotides of SEQ ID NO:27, and in particular the sequences SEQ ID NO:21-26, and more particularly the following pairs of primers:

. the sequences SEQ ID NO:21 and SEQ ID NO:22, corresponding to positions 295-314 and 485-504, respectively, of the sequence SEQ ID NO:27;

- the sequences SEQ ID NO:23 (positions 280-299 of the sequence SEQ ID NO:27), and SEQ ID NO:22;

- the sequences SEQ ID NO:24 (positions 131-150 of the sequence SEQ ID NO:27) and SEQ ID NO:22;

- the sequences SEQ ID NO:25 (positions 295-314 of the sequence SEQ ID NO:27) and SEQ ID NO:22;

- the sequences SEQ ID NO:24 and SEQ ID NO:26 (positions 322-341 of the sequence SEQ ID NO:27);

- fragments which can be used as probes: sequence SEQ ID NO:27 and the fragments consisting of 20 to 50 nucleotides of the sequence SEQ ID NO:27, and in particular the sequence SEQ ID NO:44 (positions 204-233 of the sequence SEQ ID NO:27).

The hybridization conditions for the murine probes are similar to those set out above for the human sequences.

Given the data available to the inventors, it was not obvious that mammals might express a urotensin II and that the latter might effectively exert effects other than cardiovascular effects.

Said polypeptides can be produced either by expressing the nucleic acid sequences as defined above in host cells, or by synthesis, and in particular by synthesis according to the Merrifield technique.

A first application of the nucleic acid sequences defined above is to detect either the presence or absence of mRNA encoding a mammalian urotensin II, and in particular human urotensin II, in biological samples (biopsies, for example), especially in individuals with a neurodegenerative pathology or a trauma to the spinal cord, or to detect a mutation in the sequence of the gene, or of the mRNA, encoding urotensin (comparison with the nucleic acid sequences according to the invention).

A second application of the nucleic acid sequences defined above is the production of vectors capable of expressing the precursors of human urotensin II, in particular in the context of targeted gene therapy.

In the context of these applications, the nucleic acid sequences are advantageously selected from the group consisting of the human sequences SEQ ID NO:4 to SEQ ID NO:6, the rat sequences SEQ ID NO:18 to SEQ ID NO:20 and the mouse sequences SEQ ID NO:27 to SEQ ID NO:29.

A subject of the present invention is also a cell transformed with at least one nucleic acid fragment as defined above.

A subject of the present invention is also pharmaceutical compositions, characterized in that they comprise at least one polypeptide as defined above or at least one nucleic acid sequence encoding all or part of said polypeptides, combined with at least one pharmaceutically acceptable vehicle.

For the purpose of the present invention, the term "pharmaceutically acceptable vehicle" is intended to mean both conventional vehicles and those used in the context of gene therapy.

Preferably, said compositions are administered intrathecally.

The compositions according to the present invention make it possible, in particular, to treat neurodegenerative diseases of the spinal cord, in particular diseases of the neuromuscular end-plate, and more particularly amyotrophic diseases, such as amyotrophic lateral sclerosis, or traumas to the spinal cord, more particularly paraplegias and hemiplegias.

In an advantageous embodiment of the invention, said compositions are characterized in that the polypeptide is chosen from the group consisting of human prepro-urotensin II (SEQ ID NO:1), human pro-urotensin II (SEQ ID NO:2) and human urotensin II (SEQ ID NO:3), rat prepro-urotensin II (SEQ ID NO:30), rat pro-urotensin II (SEQ ID NO:31) and rat urotensin II (SEQ ID NO:32), and mouse prepro-urotensin II (SEQ ID NO:33), mouse pro-urotensin II (SEQ ID NO:34) and mouse urotensin II (SEQ ID NO:35).

In another advantageous embodiment of the invention, said compositions are characterized in that the polynuleotides are selected from the group consisting of the human sequences SEQ ID NO:4 to SEQ ID NO:6, the rat sequences SEQ ID NO:18 to SEQ ID NO:20 and the mouse sequences SEQ ID NO:27 to SEQ ID NO:29.

A subject of the present invention is also the use of polypeptides belonging to the urotensin II family, or of nucleic acids encoding said polypeptides, for preparing a medicinal product intended to treat neurodegenerative diseases of the spinal cord or traumas to the spinal cord.

The polypeptides belonging to the urotensin II family, which can be used in accordance with the invention can originate both from invertebrates and vertebrates, in particular mammals, and preferably human mammals.

In an advantageous embodiment of the invention, said use is characterized in that the polypeptide is

chosen from the group consisting of human prepro-urotensin II (SEQ ID NO:1), human pro-urotensin II (SEQ ID NO:2) and human urotensin II (SEQ ID NO:3), rat prepro-urotensin II (SEQ ID NO:30), rat pro-urotensin II (SEQ ID NO:31) and rat urotensin II (SEQ ID NO:32), and mouse prepro-urotensin II (SEQ ID NO:33), mouse pro-urotensin II (SEQ ID NO:34) and mouse urotensin II (SEQ ID NO:35).

In another advantageous embodiment of the invention, said use is characterized in that the polynucleotides are selected from the group consisting of the human sequences SEQ ID NO:4 to SEQ ID NO:6, the rat sequences SEQ ID NO:18 to SEQ ID NO:20 and the mouse sequences SEQ ID NO:27 to SEQ ID NO:29.

A subject of the present invention is also a diagnostic kit, characterized in that it comprises at least one sequence as claimed in the invention, capable of detecting the presence of an mRNA, possibly modified, encoding a mammalian urotensin II, in a biological sample.

A subject of the present invention is also the use of said polypeptides, which also have hypertensive activity, for selecting antagonists of this activity (selection of antihypertensives having activity against urotensins II as claimed in the invention).

Besides the preceding arrangements, the invention also comprises other arrangements, which will emerge from the following description, which refers to examples of implementation of the process which is the subject of the present invention, and also to the attached drawings, in which:

- Figure 1 illustrates the alignment of the deduced amino acid sequences of, respectively, human, frog and carp prepro-UII. In this figure, the signal sequence is indicated in italics; the conserved amino acids are indicated in black; the cleavage sites of the prohormone are indicated by stars and the conserved amino acid residues are indicated by a black circle. The disulfide bridge present in the UII sequence is

indicated under the urotensin II sequence. The amino acids are numbered on the right of the figure;

- Figure 2 illustrates the structure of human prepro-UII, pro-UII and UII;

- Figure 3 illustrates the structure of rat prepro-UII, pro-UII and UII;

- Figure 4 illustrates the structure of mouse prepro-UII, pro-UII and UII;

- Figure 5 illustrates the tissue distribution of human prepro-UII mRNA. Figure 5A illustrates the *dot blot* analysis of the expression of prepro-UII mRNA in various human tissues, using the Clontech Masterblot (poly(A) RNA from 50 different human tissues (80-448 ng/point, standardized using the level of RNA expression of 8 housekeeping genes). The positive controls consist of human genomic DNA; the negative controls include DNA or RNA from yeast or from *E. coli*, and also human repeat genomic sequences (H). The *blot* is hybridized with the probe of cDNA encoding human prepro-UII, and exposed to an X-Omat film for 2 days. Figure 5B illustrates the Northern Blot analysis of the expression of prepro-UII mRNA in the human spinal cord; 2 µg of spinal cord poly(A) mRNA are hybridized with the probe consisting of the human prepro-UII cDNA. The size is determined using RNA size markers (calibrated standard nucleotide chains). Figure 5C corresponds to X-ray autoradiographs and shows the distribution of prepro-UII mRNA in the human spinal cord. The frontal sections are hybridized with an antisense (1) or sense (2) prepro-UII riboprobe, and exposed to X-ray-sensitive films for 10 days;

- Figure 6 is a comparison of the primary structures of urotensin II from various species. Dashes have been inserted in order for the sequences to be optimally aligned. The dots illustrate the amino acids residues which are identical between the various sequences, with respect to the human sequence;

- Figure 7 illustrates the tissue distribution of rat and of mouse prepro-UII mRNA.

It should be clearly understood, however, that these examples are given merely as an illustration of the subject of the invention, of which they in no way constitute a limitation.

EXAMPLE

- *Materials and methods*

* Isolation of the human prepro-UII cDNA:

An EST (*expressed sequence tag*) sequence encoding a peptide having a certain identity with frog urotensin II is registered under the no. AA535545 (Genbank). This sequence derives from an EST analysis of cDNA clones obtained from colon tumors.

Two primers (5'-AACCCAAGAGGAAATTTGAGAAAGTT-3' (SEQ ID NO:7) and 5'-CCAGGTAACAATGAACAGGGTGTAG-3' (SEQ ID NO:8)) deduced from the EST sequence enable a 269 bp fragment to be synthesized by RT-PCR from a human colon tumor sample, under the following conditions:

94°C, 4 min, 1X; 94°C, 1 min; 55°C, 1 min; 72°C, 1 min, 30X; 72°C, 5 min, 1X.

The PCR product is labeled with [^{32}P] dCTPs by random priming, and then hybridized with various human tissues containing poly(A) RNAs and also with positive and negative controls (MasterBlot, Clontech, Palo Alto). The hybridization and washes are carried out under the following conditions:

* prehybridization: incubation at 42°C, at least 5 hours in a reaction medium comprising:

50% formamide, 5X SSC, 5X Denhardt's, 50 mM NaH_2PO_4/Na_2HPO_4, 200 µg/ml salmon sperm DNA, 0.1% SDS.

* hybridization: the same medium as the prehybridization medium, with the labeled probe in addition.

* washes: 4 times 5 minutes at room temperature, 2X SSC + 0.1% SDS, and then twice 10 minutes at 42°C, 0.1% SDS + 0.1% SDS.

The *blot* is exposed against an X-OMAT film (Kodak) and the hybridization signals are quantified using the Densylab program (Bioprobe Systems, France).

The strongest hybridization signal is obtained in the spinal cord.

Under these conditions, poly(A) RNA from human spinal cord (Clontech) is used to amplify the 5' end of the human UII cDNA using a RACE kit (Marathon cDNA amplification kit, Clontech).

* *Northern blot* analysis (RNA transfer onto membrane):

2 µg of poly(A) RNA from human spinal cord (Clontech) are loaded onto an agarose-formaldehyde gel; after migration and transfer onto nylon membrane, hybridization is carried out with the PCR product specific for the human UII cDNA, labeled by incorporation of [^{32}P] dCTP.

* *In situ* hybridization:

Sense and antisense human riboprobes are prepared by *in vitro* transcription of the PCR products obtained with specific prepro-UII primers, 5'-CTGCCAGAGATGCTGGGTG-3' (SEQ ID NO:10) and 5'-GACACAGTATTTCCAGAAGCAATC-3' (SEQ ID NO:11), extended at their 5'-terminal end with the SP6 and T7 promoters of the corresponding RNA polymerases; the transcription is carried out in the presence of [^{35}S]UTP (Amersham) or of digoxigenin-11-UTP (Boehringer), and of T3 or T7 RNA polymerase, under the same PCR conditions as those set out above.

A portion of human cervical spinal cord was obtained by autopsy from a 70-year-old male.

The tissue fragment is fixed in 4% formaldehyde for 24 hours, embedded in Tissue-Tek and frozen in liquid nitrogen.

Frontal sections (12 µm thick) are cut using a cryostat and stored at -80°C.

The sections are pretreated as described in H. Tostivint et al. (14) and covered with a prehybridization buffer (50% formamide, 0.6 M NaCl, 10 mM Tris-HCl, pH 7.5, 0.02% Ficoll, 0.02% polyvinylpyrrolidine, 0.1% BSA, 1 mM EDTA, pH 8.0,

550 µg/ml of denatured salmon sperm DNA and 50 µg/ml of yeast tRNA).

The hybridization is carried out at 55°C overnight in the same buffer (with the exception of the concentration of denatured salmon sperm DNA: 60 µg/ml), supplemented with 10 mM of dithiothreitol, 10% dextran sulfate and heat-denatured riboprobes.

The ^{35}S-labeled probes and the digoxigenin-labeled probes are diluted in the hybridization buffer so as to obtain a final concentration of $5x10^6$ dmp/ml and 1:100 (v/v), respectively.

The sections are washed in 2X SSC buffer at 60°C and treated with RNase A (50 µg/ml) for 60 min at 37°C.

Five washes under stringent conditions are carried out in a 0.1X SSC, 14 mM β-mercaptoethanol, 0.05% sodium pyrophosphate buffer at 60°C.

The sections hybridized with the ^{35}S-labeled riboprobes are dehydrated in solutions of ethanol comprising increasing concentrations of 0.3 M sodium acetate, and exposed on a Hyperfilm-βmax film (Amersham) for 2 weeks.

The sections hybridized with the digoxigenin-labeled riboprobes are washed in a buffer 1 (100 mM Tris-HCl and 150 mM NaCl, pH 7.5), incubated for 30 min in a blocking buffer (2% of Boehringer blocking agent in buffer 1) and incubated for 2 hours in buffer 1 containing 1:500 of alkaline phosphate-conjugated anti-digoxigenin antibodies (Boehringer), 1% of normal sheep serum and 0.1% of Triton X100. The sections are rinsed twice, for 10 min in buffer 1 and 10 min in buffer 2 (100 mM Tris-HCl, 100 mM NaCl and 50 mM $MgCl_2$, pH 9.5), and then incubated for 3 hours in a chromagenic solution consisting of Fast Red TR/Naphthol AS-MX and 3 mM Levamisole (Sigma).

The reaction is stopped by rinsing in a TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

The sections are examined under a microscope (Leitz Orthoplan).

* Sequencing

The amplification product is subcloned into a pGEM-T vector (Promega) and sequenced with the SP6 and T7 primers using the Amersham sequencing kit (Thermo Sequenase).

- *Results*

* Characterization of the human prepro-UII cDNA:

The open reading frame of the human UII precursor cDNAs encodes a 124 amino acid protein (Figure 1 and Figure 2).

The organization of the human UII precursors is similar to that of the carp UII prohormone and to that of the frog UII precursor. All these precursors comprise an N-terminal signal sequence and then a flanking peptide, a proteolytic cleavage site (Lys/Arg-Lys-Arg) and the urotensin II sequence located at the C-terminal end of each precursor.

The N-terminal flanking peptides of the carp, frog and human precursors exhibit virtually no similarity.

The human UII comprises only 11 amino acids, whereas the frog and carp UII have 13 and 12 amino acids, respectively (Figure 6).

The sequence of the C-terminal cyclic heptapeptide of urotensin II is conserved in the frog and in humans. On the other hand, the N-terminal region of the peptide is very variable.

In the frog, as in the carp, the C-terminal region of the flanking peptide contains a dibasic potential cleavage site (Arg-Lys and Arg-Arg) which might generate the conserved dipeptide Gln-Phe.

However, in humans, the sequence of the corresponding dipeptide is totally different (Pro-Tyr) (Figure 1 and Figure 2).

* Distribution of the human prepro-UII mRNA was studied:

The tissue distribution of the human prepro-UII mRNA was studied by dot blot analysis (Figure 5A).

Out of the 50 different tissues tested, the spinal cord shows the strongest hybridization signal. The prepro-UII mRNA is also observed in the *medulla oblongata*, but the strength of the signal is much weaker than that obtained in the spinal cord.

In the peripheral tissues, the presence of prepro-UII mRNA is detected in the kidney, spleen, small intestine, thymus, prostate, hypophysis and adrenal gland, and in smaller amounts, in the stomach, pancreas, ovaries and liver (Figure 5A).

The analysis by *Northern blot* reveals the presence of a single band corresponding to a prepro-UII mRNA of approximately 700 bp in the human spinal cord.

The labeling of sections of the cervical portion of the human spinal cord by *in situ* hybridization shows that the prepro-UII mRNA is located in the motoneurons (Figure 5C).

* Characterization of the rat, and of the mouse, prepro-UII cDNA:

The open reading frame of the rat and mouse UII precursor cDNAs encodes a 123 amino acid protein (Figures 3 and 4).

Figure 7 illustrates the results of the distribution in various rat and mouse tissues, using RT-PCR. The total RNAs are extracted and subjected to an RT-PCR reaction, under conditions similar to those set out above.

In Figure 7A, the rat (left) and mouse (right) PCR products are detected by hybridization with an internal oligonucleotide probe specific for rat and for mouse (the sequences SEQ ID NO:43 and 44, respectively).

Figure 7B illustrates GADPH PCR products, used as a control to reflect equivalent RNA levels, loaded onto an agarose gel.

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As emerges from the above, the invention is in no way limited to its methods of implementation, preparation and application which have just been described more explicitly; on the contrary, it encompasses all of the variants thereof which may occur to a person skilled in the art, without departing from the context or scope of the present invention.

CLAIMS

1) A polypeptide, isolated from mammals, characterized in that it comprises, at its C-terminal end, a heptapeptide having the following sequence: Cys-Phe,Trp-Lys-Tyr-Cys-Xaa, in which Xaa represents Val or Ile, in that it belongs to the urotensin II family and in that it exhibits at least 45%, and preferably at least 70%, similarity with the polypeptide sequence SEQ ID NO:1, corresponding to human prepro-urotensin II.

2) The mammalian polypeptide as claimed in claim 1, characterized in that it is selected from the group consisting of the human sequences SEQ ID NO:1-3, of the rat sequences SEQ ID NO:30-32 and of the mouse sequences SEQ ID NO:33-35.

3) A purified nucleic acid fragment, characterized in that it comprises all or part of a sequence encoding a polypeptide as claimed in claim 1 or claim 2, or of the sequence complementary thereto, which may be a sense or antisense sequence, with the exception of the EST having the Gen Bank accession number AA535545.

4) The nucleic acid fragment as claimed in claim 3, characterized in that it is selected from the group consisting of the sequences SEQ ID NO:4-6, the sequences SEQ ID NO:18-20 and the sequences SEQ ID NO:27-29.

5) A recombinant vector, characterized in that it contains a nucleic acid fragment as claimed in claim 3 or claim 4.

6) A cell transformed with at least one nucleic acid fragment as claimed in claim 3 or claim 4.

7) A reagent for detecting a nucleic acid fragment as claimed in claim 3 or claim 4, characterized in that it comprises between 20 and 50 nucleotides of the sequence SEQ ID NO:4, of the sequence SEQ ID NO:18 or of the sequence SEQ ID NO:27.

8) The reagent as claimed in claim 7, characterized in that it is selected from the group consisting of:

- a fragment of the sequence encoding human prepro-urotensin II, which encodes a dipeptide (Pro-Tyr), and which is upstream of the tribasic cleavage site, itself located just upstream of the sequence encoding human urotensin II and specific for said human sequence;

- fragments which can be used as primers: SEQ ID NO:7 and NO:8, SEQ ID NO:10-17; SEQ ID NO:21-26; SEQ ID NO:36-42, and

- fragments which can be used as probes: sequence SEQ ID NO:4 and the fragments consisting of 20 to 50 nucleotides of said sequence SEQ ID NO:4; sequence SEQ ID NO:18 and the fragments consisting of 20 to 50 nucleotides of said sequence SEQ ID NO:18, and sequence SEQ ID NO:27 and the fragments consisting of 20 to 50 nucleotides of said sequence SEQ ID NO:27.

9) A pharmaceutical composition, characterized in that it comprises at least one polypeptide as claimed in either of claims 1 and 2, or one nucleic acid sequence as claimed in either of claims 3 and 4 encoding all or part of said polypeptides, combined with at least one pharmaceutically acceptable vehicle.

10) The use of polypeptides belonging to the urotensin II family, or of nucleic acid sequences encoding said polypeptides, for preparing a medicinal product intended to treat neurodegenerative diseases of the spinal cord or traumas to the spinal cord.

11) A process for detecting the presence or absence of an mRNA encoding a mammalian urotensin II, in particular in individuals with a neurodegenerative pathology or a trauma to the spinal cord, by bringing a suitably treated biological sample into contact with at least one reagent as claimed in claim 7 or claim 8.

12) A process for detecting a mutation in the sequence of the gene or of the mRNA encoding urotensin, characterized in that it comprises extracting said DNA

or said mRNA from a biological sample and comparing it with the nucleic acid sequences as claimed in claim 3 or claim 4.

13) A diagnostic kit, characterized in that it comprises at least one sequence as claimed in either of claims 3 and 4, capable of detecting the presence of an mRNA, possibly modified, encoding a mammalian urotensin II, in a biological sample.

14) The use of the polypeptides as claimed in claim 1 or claim 2, for selecting anti-hypertensives.

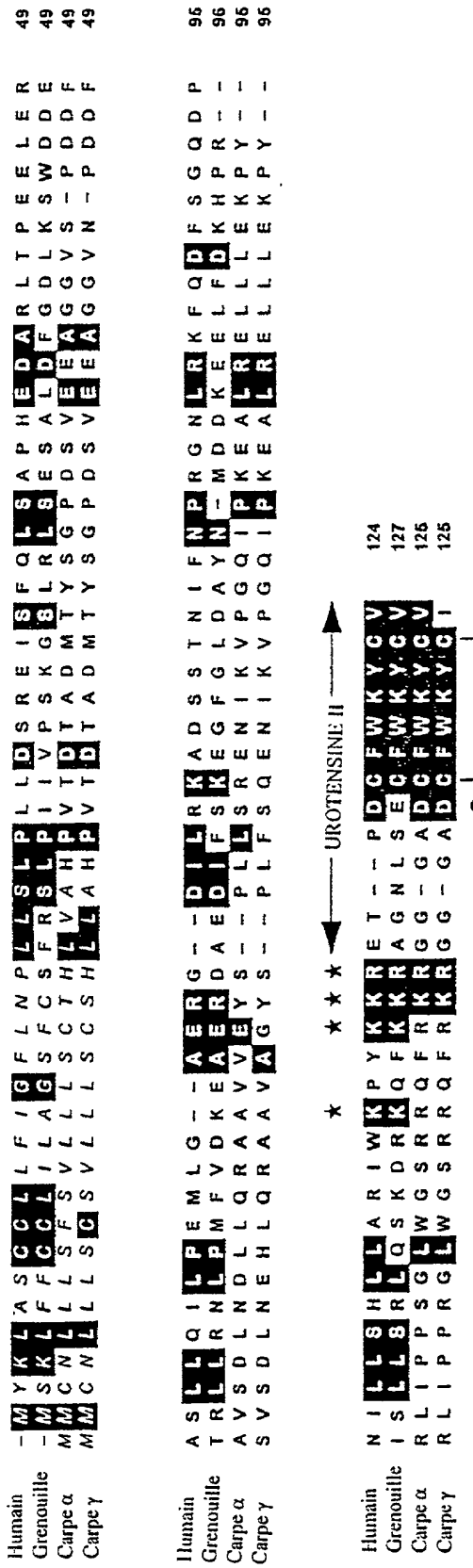

Humain
Grenouille
Carpe α
Carpe γ
UROTENSINE II
49
96
124
127
126
125

FIGURE 1

CCAAGAAGGAAGCCGTCTATCTTGTGGCGATC

ATG TAT AAG CTG GCC TCC TGC TGT TTG CTT TTC ATA GGA TTC TTA
Met Tyr Lys Leu Ala Ser Cys Cys Leu Leu Phe Ile Gly Phe Leu

PEPTIDE SIGNAL

AAT CCT CTC TTA TCT CTT CCT CTC CTT GAC TCC AGG GAA ATA TCC
Asn Pro Leu Leu Ser Leu Pro Leu Leu Asp Ser Arg Glu Ile Ser

TTT CAA CTC TCA GCA CCT CAT GAA GAC GCG CGC TTA ACT CCG GAG
Phe Gln Leu Ser Ala Pro His Glu Asp Ala Arg Leu Thr Pro Glu

PRO-SEGMENT

GAG CTA GAA AGA GCT TCC CTT CTA CAG ATA CTG CCA GAG ATG CTG
Glu Leu Glu Arg Ala Ser Leu Leu Gln Ile Leu Pro Glu Met Leu

GGT GCA GAA AGA GGG GAT ATT CTC AGG AAA GCA GAC TCA AGT ACC
Gly Ala Glu Arg Gly Asp Ile Leu Arg Lys Ala Asp Ser Ser Thr

AAC ATT TTT AAC CCA AGA GGA AAT TTG AGA AAG TTT CAG GAT TTC
Asn Ile Phe Asn Pro Arg Gly Asn Leu Arg Lys Phe Gln Asp Phe

TCT GGA CAA GAT CCT AAC ATT TTA CTG AGT CAT CTT TTG GCC AGA
Ser Gly Gln Asp Pro Asn Ile Leu Leu Ser His Leu Leu Ala Arg

ATC TGG AAA CCA TAC AAG AAA CGT GAG ACT CCT GAT TGC TTC TGG
Ile Trp Lys Pro Tyr Lys Lys Arg Glu Thr Pro Asp Cys Phe Trp

UROTENSINE II

AAA TAC TGT GTC TGA
Lys Tyr Cys Val ***

AGTGAAATAAGCATCTGTTAGTCAGCTCAGAAACACCCATCTTAGAATATGAAAAATAACACA
ATGCTTGATTTGAAAACAGTGTGGAGAAAAACTAGGCAAACTACACCCTGTTCATTGTTACCT
GGAAAATAAATCCTCTAT

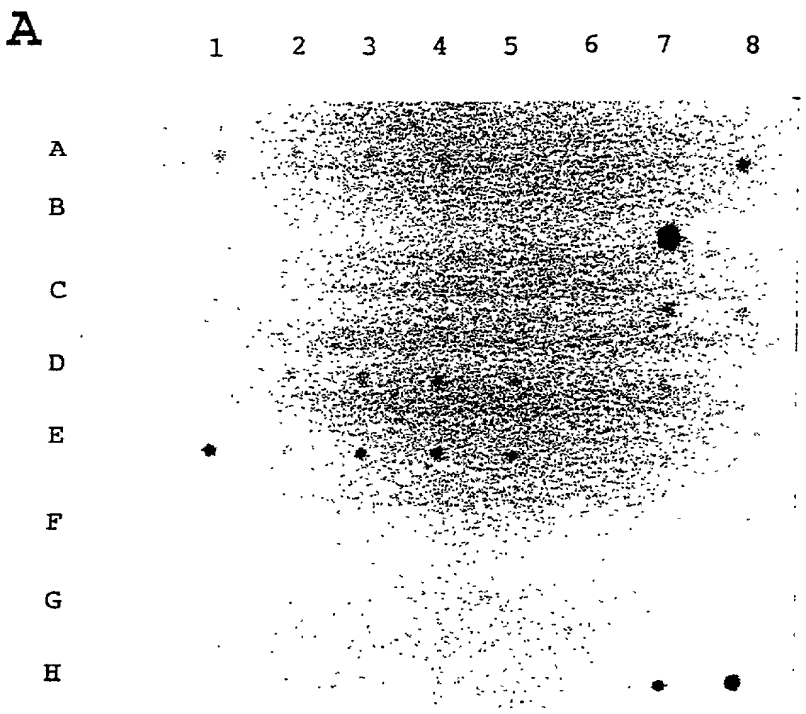
FIGURE 2

```
                9          18         27         36         45         54
5'  CGG AGC AGA CAC CCA GCC AGA CTT CTT CCC GTC GTC ATG GAC AGG GTG CCC TTC
                                                 Met Asp Arg Val Pro Phe
                                                 ◄.....................

               63         72         81         90         99        108
    TGC TGC CTG CTC TTC GTA GGA CTC CTG AAT CCA CTC CTG TCT TTT CCC GTC ACG
    Cys Cys Leu Leu Phe Val Gly Leu Leu Asn Pro Leu Leu Ser Phe Pro Val Thr
    .....................................................►.....  .....

                          peptide signal

              117        126        135        144        153        162
    GAC ACT GGT GAA ATG TCT CTT CAG CTT CCA GTG CTT GAG GAA AAT GCT CTT CGG
    Asp Thr Gly Glu Met Ser Leu Gln Leu Pro Val Leu Glu Glu Asn Ala Leu Arg
    ..........................................................................

              171        180        189        198        207        216
    GCT CTG GAG GAG CTG GAG AGG ACT GCC CTC CTG CAG ACG CTG CGC CAG ACC GTG
    Ala Leu Glu Glu Leu Glu Arg Thr Ala Leu Leu Gln Thr Leu Arg Gln Thr Val
    ..........................................................................

                          pro-segment

              225        234        243        252        261        270
    GGC ACA GAA GCA GAG GGA AGC CTT GGC CAG GCA GAT CCC AGT GCC GAG ACT CCC
    Gly Thr Glu Ala Glu Gly Ser Leu Gly Gln Ala Asp Pro Ser Ala Glu Thr Pro
    ..........................................................................

              279        288        297        306        315        324
    ACT CCA AGG GGA AGC TTG AGG AAG GCT CTC ACT GGG CAA GAT TCT AAC ACT GTA
    Thr Pro Arg Gly Ser Leu Arg Lys Ala Leu Thr Gly Gln Asp Ser Asn Thr Val
    ..........................................................................

              333        342        351        360        369        378
    CTG AGC CGT CTT TTG GCG AGA ACC AGG AAA CAA CGT AAG CAA CAC GGG ACT GCC
    Leu Ser Arg Leu Leu Ala Arg Thr Arg Lys Gln Arg Lys Gln His Gly Thr Ala
    ..........................................................  ◄.........

              387        396        405        414        423        432
    CCA GAA TGC TTC TGG AAG TAC TGC ATT TCA AGA GAG ACG TCT CCT CAG AAC CAT
    Pro Glu Cys Phe Trp Lys Tyr Cys Ile ***
    ◄.......  ◄.......  ◄.....►

                          UrotensineII

              441        450        459        468        477        486
    CAC TTC AGG AAA CTA AAG AGC AGA TGC TTG AAG AAA AAT CGT GCC AAC AAC GCC

              495        504        513        522
    CCG TTC TCC ACT ATG AGA AAT AAA CCC TCT ATG TTT CTC AAC T 3'
```

FIGURE 3

```
            9          18          27          36          45          54
5'  CCA GAG CAG ACG CCC AGA CGG ACT TCT CGC CGC ATC ATG GAC AGG GTG CCC TTC
                                                Met Asp Arg Val Pro Phe
                                                <-----------------------

           63          72          81          90          99         108
TGC TGC CTG CTC TTC ATA GGA CTT CTG AAT CCA CTG CTG TCC CTT CCC GTC ACG
Cys Cys Leu Leu Phe Ile Gly Leu Leu Asn Pro Leu Leu Ser Leu Pro Val Thr
.....................................................>  <............
                        peptide signal

          117         126         135         144         153         162
GAC ACT GGT GAG AGG ACT CTT CAG CTT CCA GTG CTT GAG GAA GAC GCT CTT CGG
Asp Thr Gly Glu Arg Thr Leu Gln Leu Pro Val Leu Glu Glu Asp Ala Leu Arg
.....................................................................

          171         180         189         198         207         216
GCT CTG GAG GAG CTG GAG AGG ATG GCC CTC CTG CAG ACC CTG CGT CAG ACC ATG
Ala Leu Glu Glu Leu Glu Arg Met Ala Leu Leu Gln Thr Leu Arg Gln Thr Met
.....................................................................
                              pro-segment

          225         234         243         252         261         270
GGC ACG GAA GCA GGG GAG AGC CCT GGA GAA GCA GGT CCC AGC ACT GAG ACT CCC
Gly Thr Glu Ala Gly Glu Ser Pro Gly Glu Ala Gly Pro Ser Thr Glu Thr Pro
.....................................................................

          279         288         297         306         315         324
ACT CCA CGG GGA AGC ATG AGG AAG GCT TTC GCT GGG CAA AAT TCT AAC ACT GTA
Thr Pro Arg Gly Ser Met Arg Lys Ala Phe Ala Gly Gln Asn Ser Asn Thr Val
.....................................................................

          333         342         351         360         369         378
CTG AGT CGT CTC TTG GCA AGA ACC AGG AAA CAA CAT AAG CAA CAC GGG GCT GCC
Leu Ser Arg Leu Leu Ala Arg Thr Arg Lys Gln His Lys Gln His Gly Ala Ala
.....................>                <-----------------------------------

          387         396         405         414         423         432
CCA GAG TGC TTC TGG AAA TAC TGC ATT TGA GGA GAC ACA AGC GCC CGT TGG TCT
Pro Glu Cys Phe Trp Lys Tyr Cys Ile ***
<-------------------------------------
                    Urotensine II

          441         450         459         468         477         486
CTC AGA ACC ATT ACA TTC AGG AAA CGG GCA GAG CAG ATG CTT GAA GCA AAA TCA

          495         504         513         522         531
CGC TAA CGA CGC CTT GTT CTT CAT TAT GAG AAA TAA ATC CTC TAT GTT TCT CA  3'
```

FIGURE 4



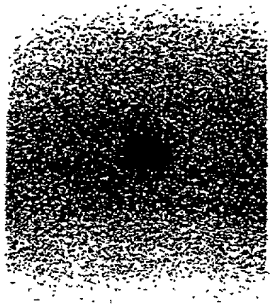
	1	2	3	4	5	6	7	8
A	cerveau entier	amygdale	noyau caudé	cervelet	cortex cérébral	lobe frontal	hippocampe	*medulla oblongata*
B	lobe occipital	putamen	*locus niger*	lobe temporal	thalamus	noyau sous-thalamique	moelle épinière	-
C	cœur	aorte	muscle squelettique	colon	vessie	utérus	prostate	estomac
D	testicules	ovaires	pancréas	hypo-physe	glande surrénale	thyroïde	glande salivaire	glande mammaire
E	rein	foie	intestin grêle	rate	thymus	leucocyte périphérique	ganglion lymphatique	moelle osseuse
F	appendice	poumon	trachée	placenta	-	-	-	-
G	cerveau foetal	cœur foetal	rein foetal	foie foetal	rate foetale	thymus foetal	poumon foetal	-
H	ARN total de levure 100 ng	ARNt de levure 100 ng	ARNr d'*E. coli* 100 ng	ADN d'*E. coli* 100 ng	poly r(A) 100 ng	ADN C_0t1 humain	ADN humain 100 ng	ADN humain 500 ng

FIGURE 5.1

B

moelle épinière

725 pb →



C

(1)

(2)

FIGURE 5.2

WO 00/31265

09/831907
PCT/FR99/02941

Humain	E	-	T	P	-	D	C	F	W	K	Y	C	V
Grenouille	A	G	N	L	S	E	.	.	.	.	.	.	.
Goujon	A	G	.	A	-	.	.	.	.	.	.	.	.
Truite	G	G	N	S	-	E	.	.	.	.	.	.	.
Poisson ventouse A	G	S	G	A	-	.	.	.	.	.	.	.	.
Poisson ventouse B	G	S	N	T	-	E	.	.	.	.	.	.	.
Carpe α	G	G	G	A	-	.	.	.	.	.	.	.	.
Carpe β1	G	G	N	T	-	E	.	.	.	.	.	.	.
Carpe β2	G	S	N	T	-	E	.	.	.	.	.	.	.
Carpe γ	G	G	G	A	-	.	.	.	.	.	.	.	.
Flet	A	G	.	T	-	E	.	.	.	.	.	.	I
Esturgeon	-	G	S	T	S	E	.	.	.	.	.	.	.
Poisson spatule	-	G	S	T	S	E	.	.	.	.	.	.	.
Raie	N	N	F	-	S	.	.	.	.	.	.	.	.
Roussette	N	N	F	-	S	.	.	.	.	.	.	.	.
Lamproie	N	N	F	-	S	.	.	.	.	.	.	.	.

FIGURE 6

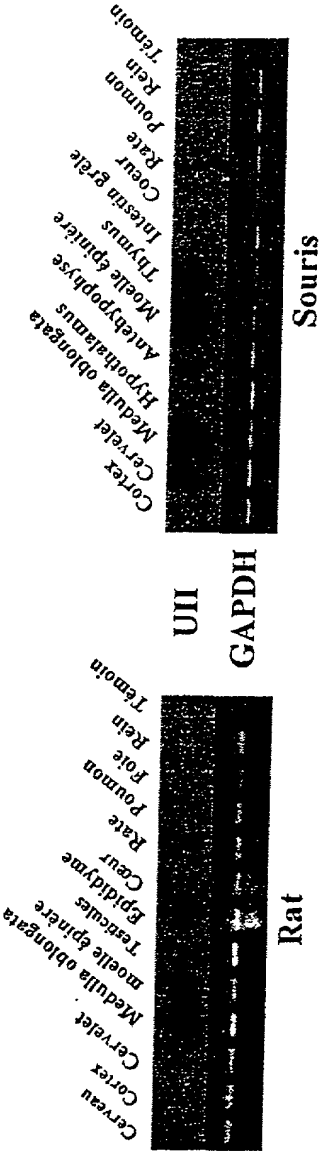


FIGURE 7

Declaration and Power of Attorney for Patent Application
Déclaration et Pouvoirs pour Demande de Brevet
French Language Declaration

En tant l'inventeur nommé ci-après, je déclare par le présent acte que :

Mon domicile, mon adresse postale et ma nationalité sont ceux figurant ci-dessous à côté de mon nom.

Je crois être le premier inventeur original et unique (si un seul nom est mentionné ci-dessous), ou l'un des premiers co-inventeurs originaux (si plusieurs noms sont mentionnés ci-dessous) de l'objet revendiqué, pour lequel une demande de brevet a été déposée concernant l'invention intitulée

et dont la description est fournie ci-joint à moins

☐ ci-joint

☐ a été déposée le

sous le numéro de demande des Etats-Unis ou le numéro de demande international PCT

et modifiée le

(le cas échéant).

Je déclare par le présent acte avoir passé en revue et compris le contenu de la description ci-dessus, revendications comprises, telles que modifiées par toute modification dont il aura été fait références ci-dessus.

Je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations.

As a below named inventor, I hereby declare that :

My residence, post office address and citizenship are as stated next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed an for which a patent is sought on the invention entitled

UROTENSINS II OF MAMMALS AND THEIR USES

the specification of which :

☐ is attached hereto.

☒ was filed on

as United States Application Number or PCT International Application Number.
PCT/FR99/02941 filed on November 26, 1999

and was amended on

(if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations,§ 1.56.

French Language Declaration

Je revendique par le présent acte avoir la priorité étrangère, en vertu du Titre 35, § 119(a)-(d) ou § 365(b) du Code des Etats-Unis, sur toute demande étrangère de brevet ou certificat d'inventeur ou, en vertu du Titre 35, § 365(a) du même Code, sur toute demande internationale PCT désignant au moins un pays autre que les Etats-Unis et figurant ci-dessous et, en cochant la case, j'ai aussi indiqué ci-dessous toute demande étrangère de brevet, tout certificat d'inventeur ou toute demande internationale PCT ayant date de dépôt précédant celle de la demande à propos de laquelle une priorité est revendiquée.

I hereby claim foreign priority under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below, and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign application(s)
Demande(s) de brevet antérieure(s) dans un autre pays.

(Number) (Numéro)	(Country) (Pays)	(Day/Month/Year Filed) (Jour/Mois/Anné de dépôt)	Priority claimed Droit de priorité revendiqué ☒ Yes / Oui	☐ No / Non
98/14914	**FRANCE**	**26/11/1998**		
(Number) (Numéro)	(Country) (Pays)	(Day/Month/Year Filed) (Jour/Mois/Anné de dépôt)	☐ Yes / Oui	☐ No / Non

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 119(e) du Code des Etats-Unis, de toute demande de brevet provisoire effectuée aux Etats-Unis et figurant ci-dessous.

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 120 du Code des Etats-Unis, de toute demande de brevet effectuée aux Etats-Unis, ou en vertu du Titre 35, § 365© du même Code, de toute demande internationale PCT désignant les Etats-Unis et figurant ci-dessous et, dans la mesure où l'objet de chacune des revendications de cette demande de brevet n'est pas divulgué dans la demande antérieure américaine ou internationale PCT, en vertu des dispositions du premier paragraphe du Titre 35, § 112 du code des Etats-Unis, je reconnais devoir divulguer toute information pertinente à la brevetabilité, comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations, dont j'ai pu disposer entre la date de dépôt de la demande antérieure et la date de dépôt de la demande nationale ou internationale PCT de la présente demande :

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)	(Status) (patented, pending,abandoned) (Statut) (breveté, en cours d'examen, abandonné)
(Application No.) (N° de demande)	(Filing Date) (Date de dépôt)	(Status) (patented, pending,abandoned) (Statut) (breveté, en cours d'examen, abandonné)

Je déclare que par le présent acte que toute déclaration ci-incluse est, à ma connaissance, véridique et que toute déclaration formulée à partir de renseignements ou de suppositions est tenue pour véridique ;et de plus, que toutes ces déclarations ont été formulées en sachant que toute fausse déclaration volontaire ou son équivalent est passible d'une amende ou d'une incarcération, ou des deux, en vertu de la section 1001 du Titre 18 du Code de Etats-Unis, et que de telles déclarations volontairement fausses risquent de compromettre la validité de la demande de brevet ou du brevet délivré à partir de celle-ci.

I hebery declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

French Language Declaration

POUVOIRS : En tant que l'inventeur cité, je désigne par la présente l'(les) avocats(s) et/ou agent(s) suivant(s) pour qu'ils poursuive(nt) la procédure de cette demande de brevet et traite(nt) toute affaire s'y rapportant avec l'Office des brevets et des marquees : (mentionner le nom et le numéro d'enregistrement).

POWER OF ATTORNEY : As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to persecute this application and transact all bussiness in the Patent and Trademark Office connected therewith : (list name and registration number)

Norman F.Oblon, Reg. No. 24,618 ; Marvin J. Spivak, Reg. No. 24,913 ; C. Irvin McClelland, Reg. No. 21,124 ; Gregory J. Maier, Reg.No. 25,599 ; Arthur I. Neustadt, Reg. No. 24,854 ; Richard D. Kelly, Reg. No. 27,757 ; James D. Hamilton, Reg. No. 28,421 ; Eckhard H. Kuesters, Reg. No. 28,870 ; Robert T. Pous, Reg. No. 29,099 ; Charles L. Gholz, Reg. No.26,395 ; William E. Beaumont, Reg. No. 30,996 ; Jean-Paul Lavalleye, Reg. No.31,451 ; Stephen G. Baxter, Reg. No. 34,884 ; Richard L. Treanor, Reg. No.36,379 ; Stephen P. Weihrouch, Reg. No. 32,829 ; John T. Goolkasian, Reg. No. 26,142 ; Richard L. Cinn, Reg. No. 34,305 ; Stephen E. Lipman, Reg. No. 30,011 ; Carl E. Shlier, Reg. No. 34,426 ; James J. Kubaski, Reg. No. 34,648 ; Richard A. Neifeld, Reg. No. 35,299 ; J. Dereck Mason, Reg. No. 35,270 ; Surinder Sachar, Reg. No. 34,423 ; Christina M. Gadiano, Reg. No. 37,628 ; Jeffrey B. McIntyre, Reg. No. 36,867 ; William T. Enos, Reg. No. 33,128 ; Michael E. McCabe, Jr., Reg. No. 37,182 ; Bradley D. Lytle, Reg. No. 40,073 ; and Michael R. asey, Reg. No. 40,294, with full powers of substitution and revocation.

Addresser toute correspondance à :

Send Correspondence to :

OBLON, SPIVAK, McCLELLAND, MAIER & NEUSTADT, P.C.
FOURTH FLOOR
1755 JEFFERSON DAVIS HIGHWAY
ARLINGTON, VIRGINIA 22202 U.S.A.

Adresser tout appel téléphonique à :
(nom et numéro de téléphone)

Direct Telephone calls to : (name and telephone number)

(703) 413-3000

Nom complete de l'unique ou premier inventeur **BEAUVILLAIN Jean-Claude**	Full name of sole or first inventor
Signature de l'inventeur Date 16/07/2001	Inventor's signature Date
Domicile 59175 TEMPLEMARS (France) FRX	Residence
Nationalité Française	Citizenship
Adresse Postale 47, Bis Rue Wattrelot 59175 TEMPLEMARS (France)	Post Office Address
Nom complete du second co-inventeur, le cas echeant **COULOUARN Yolaine**	Full name of second joint inventor, if any
Signature de l'inventeur Date Yolaine Coulouarn 19/07/2001	Second inventor's signature Date
Domicile 28230 EPERNON (France) FRX	Residence
Nationalité Française	Citizenship
Adresse Postale 8, Rue aux Juifs 28230 EPERNON (France)	Post Office Address

(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire.)

(Supply similar information and signature for third and subsequent joint inventors.)

French Language Declaration

Nom complete du troisième co-inventeur, le cas échéant **JEGOU Sylvie**	Full name of third joint inventor, if any
Signature de l'inventeur Date 12/04/01	Third inventor's signature Date
Domicile 76000 ROUEN (France) F-RX	Residence
Nationalité Française	Citizenship
Adresse Postale 4, Impasse Tabouret 76000 ROUEN (France)	Post Office Address
Nom complete du quatrième co-inventeur, le cas echeant **LIHRMANN Isabelle**	Full name of fourth joint inventor, if any
Signature de l'inventeur Date 31/08/01	Fourth inventor's signature Date
Domicile 27310 SAINT OUEN DE THOUBERVILLE (France) F-RX	Residence
Nationalité Française	Citizenship
Adresse Postale 19, Rue de la Haizette 27310 SAINT OUEN DE THOUBERVILLE (France)	Post Office Address
Nom complete du cinquième co-inventeur, le cas echeant **VAUDRY Hubert**	Full name of fifth joint inventor, if any
Signature de l'inventeur Date 31/08/01	Fifth inventor's signature Date
Domicile 76133 MANEGLISE (France) F-RX	Residence
Nationalité Française	Citizenship
Adresse Postale 36 Route d'Epouville 76133 MANEGLISE (France)	Post Office Address
Nom complete du sixième co-inventeur, le cas echeant	Full name of sixth joint inventor, if any
Signature de l'inventeur Date	Sixth inventor's signature Date
Domicile	Residence
Nationalité	Citizenship
Adresse Postale	Post Office Address

(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire.)

(Supply similar information and signature for third and subsequent joint inventors.)

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